renocorticotropic hormone causes sodium and potassium loss when it intensifies the glycosuria of diabetic rats, an effect opposite to that produced by growth hormone in these experiments when it enhanced the glycosuria. Posterior pituitary antidiuretic hormone might be expected to have effects upon electrolyte balance, but one would anticipate that they would be in the direction of sodium loss. The growth hormone preparation used in these experiments was assayed for antidiuretic activity by the Ham and Landis(6) procedure and found to be free of any antidiuretic activity whatsoever at a single dose level of 0.5 mg, a daily dose found to produce changes in sodium and potassium balance of normal animals (unpublished observations).

Conclusion. The administration of growth hormone to partially depancreatized diabetic rats produces a reduction in urinary nitrogen, sodium and potassium excretion, although there may be a concomitant increase in glycosuria.

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Circulating Antibodies in Vitamin-Deficiency States. Pteroylglutamic Acid, Niacin-Tryptophan, Vitamins B_{12}, A, and D Deficiencies.* (18836)

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Previous work in this laboratory has demonstrated a severe impairment of antibody response in pyridoxine and pantothenic acid-deficient rats(1). Riboflavin(1), biotin and thiamine deficiencies(2) had considerably less effect. Later experiments proved conclusively that the absence of pantothenic acid *per se* and not the concomitant inanition was responsible for antibody impairment(3). Recently we have presented evidence which suggests that pantothenic acid and pyridoxine function directly in antibody synthesis(4).

In the present paper, the relationship between vitamins and antibodies has been extended further to include the effect of deficiencies of pteroylglutamic acid, niacin-tryptophan, vit. B_{12}, vit. A, and vit. D upon the antibody response of the rat to the antigenic stimulus of human erythrocytes.

Methods. Ninety-six male weanling albino rats of the Sprague-Dawley strain were distributed into 5 groups as indicated in Table I. The animals were housed individually in wide-meshed, screen-bottom cages and weighed weekly. The composition of the basal diets fed to each group is presented in Table II. These basal diets were fed *ad lib.* throughout the experimental period. Immunization was instituted in the vit. A, B_{12}, and D groups after 4 weeks, in the niacin-tryptophan group after 5 weeks, and in the pteroylglutamic acid group after 9 weeks on experiment. A 10% suspension of washed Group O, Rh positive human erythrocytes in physiological saline was injected intraperitoneally as antigen. An initial dosage of 0.5 ml of the erythrocyte suspension was followed by two 1 ml injections on alternate days. Five days after the final injection, the rats were bled from the

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TABLE I. Summary of Growth Data, Blood Counts, and Bone Ash Analyses.*

Type	No. of rats	Body wt Initial	Body wt Final†	Blood counts† Leucocytes‡	Blood counts† Granulocytes‡	% bone ash Femurs	% bone ash Humerii
Pteroylglutamic acid							
Controls	10	39	320	19800	1700	—	—
Deficient	9	38	141	4900	100	—	—
Niacin–tryptophan							
Niacin controls	5	44	208	—	—	—	—
Tryptophan controls	5	44	189	—	—	—	—
Deficient	10	43	66	—	—	—	—
Vit. A, Controls	10	44	172	—	—	—	—
Deficient	15	44	79	—	—	—	—
Vit. B_{12}, Controls	6	45	129	—	—	—	—
Deficient	7	45	90	—	—	—	—
Vit. D, Controls	10	42	86	—	—	34.66	32.26
Deficient	9	43	73	—	—	20.24	23.78

* All values are group averages.
† At time of bleeding for antibody determination.
‡ No. per cmm of blood.

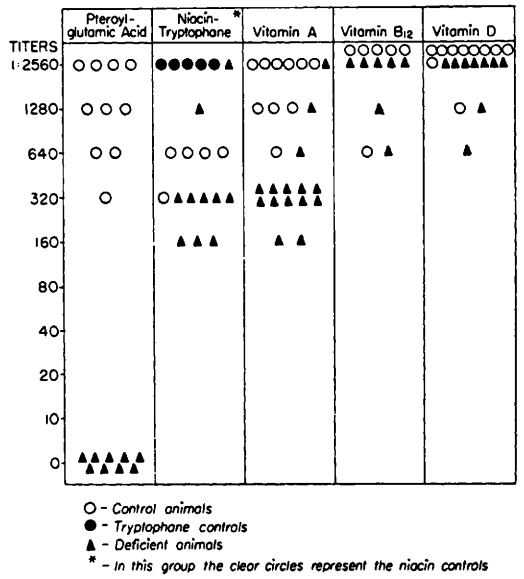
TABLE II. Composition of the Basal Diets.

Component	Pteroyl-glutamic acid†‡	Niacin-trypto-phan†§	Vit. A‖¶	Vit. B_{12}†**	Vit. D††
			(% composition)		
Casein "vitamin-free"	25	9	25	—	—
Sucrose	57.77	44.62	60.76	—	—
Hydrogenated vegetable oil	10	—	—	—	—
Corn oil	2	2	—	5	—
Cotton seed oil	—	—	10	—	—
Salts(5)	4	4	4	2	—
Sulfasuxidine	1	—	—	—	—
Choline chloride	.2	.20	.20	.10	—
i-inositol	.03	.03	.03	.02	—
dl-α-tocopherol acetate	.01	.01	.01	.01	—
2-methyl-1, 4-naphthoquinone	.001	.001	.001	.001	—
L (−) cystine	—	.15	—	.30	—
p-aminobenzoic acid	—	—	.01	.025	—
Iodinated casein	—	—	—	.10	—
Corn grits	—	40	—	—	—
Soybean meal (6% fat)	—	—	—	46.22	—
Whole ground yellow corn	—	—	—	46.22	76
Ground gluten	—	—	—	—	20
Calcium carbonate	—	—	—	—	3
Sodium chloride	—	—	—	—	1
			Daily vit. supplementation (γ)*		
Thiamine	40	40	40	160	40
Riboflavin	60	60	60	240	60
Pyridoxine	50	50	50	200	50
Calcium pantothenate	300	150	150	800	150
Niacin	100	—	100	800	100
Biotin	3	1	1	10	1
Pteroylglutamic acid	—	1	1	10	1

* Supplied in daily pill.
† All animals received 2 drops haliver oil weekly *per os* furnishing 3000 U.S.P. units of vit. A and 24 U.S.P. units of vit. D.
‡ 20 μg of pteroylglutamic acid were included in daily vit. pill of controls.
§ Daily vitamin pill of niacin controls contained 150 μg niacin. For tryptophan controls, basal diet was supplemented with 0.4% DL-tryptophan at expense of sucrose.
‖ Each rat received weekly 250 U.S.P. units of crystalline vit. D_3 (Drisdol) *per os* and 10 mg of ascorbic acid by intraperitoneal injection.
¶ Controls received weekly 2 drops Wesson oil containing 3000 U.S.P. units of vit. A acetate.
** Controls received 0.4 μg of vit. B_{12} on alternate days by intraperitoneal injection.
†† Controls received weekly *per os* 250 U.S.P. units of crystalline vit. D_3 (Drisdol).

TABLE III

INDIVIDUAL HEMAGGLUTININ TITERS



heart, under ether anesthesia and the sera tested for agglutinin titers as described previously(1). Total leucocyte determinations and differential counts were carried out in the animals of the pteroylglutamic acid group in accordance with standard procedures. Two hundred cells were counted in the differential determinations. Bone ash analyses of the femurs and humerii of the animals of the vitamin D group were made at the termination of the experiment.

Results. The individual hemagglutinin titers are recorded in Table III. It is evident that a severe impairment of antibody response occurred in pteroylglutamic acid-deficient rats. Thus, none of these animals possessed a measurable agglutinin titer. The decreased growth rate, leucocyte and granulocyte counts constitute evidence for the development of a pteroylglutamic acid deficiency state (Table I).

The results of the niacin-tryptophan experiment demonstrated that supplementation of the basal diet (deficient in both niacin and tryptophan) with either niacin or tryptophan produced a marked growth response (Table I). In contrast, however, a *normal* antibody response was obtained only in those animals receiving added tryptophan (Table III). Thus, the serum antibody content of the niacin controls did not differ significantly from that observed in the deficient animals. It is also evident that a niacin-tryptophan deficiency produced only a relatively moderate impairment of antibody response.

It may be further seen in Table III that a significant decrease in antibody levels was observed in vitamin A-deficient rats. The magnitude of this decrease, however, was not as great as that observed in pteroylglutamic acid or pantothenic acid-deficient rats, where, as a general rule the serum antibody content following the same antigenic stimulus is not detectable by our methods(1,3). Characteristic vitamin A-deficiency symptoms were observed in the animals not receiving the vitamin A supplementation.

In contrast to the above observations, no significant difference was noted between the antibody titers of vit. B_{12} or vit. D-deficient rats and those of their respective controls. A marked decrease in the percentage of bone ash was observed in the rats of the vit. D group not receiving added vit. D (Table I).

Discussion. The results of the present experiment clearly demonstrate that a severe impairment of antibody response occurred in pteroylglutamic acid-deficient rats. Similar findings were reported by Little *et al.*(6) in chicks which can be made pteroylglutamic acid-deficient without recourse to sulfa drugs. It is well recognized that a pteroylglutamic acid deficiency produces a leucopenic state. Since lymphocytes may be the site of antibody formation(7-9) it is conceivable that the suppression of circulating antibodies observed in pteroylglutamic acid-deficient rats could be attributed to the parallel development of the leucopenia. Preliminary curative experiments

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in which massive doses of pteroylglutamic acid were administered to previously immunized pteroylglutamic acid-deficient rats resulted in: (a) an immediate and sustained growth response, (b) a pronounced rise in the number of leucocytes within 7 days, and (c) no significant increase in antibody titers within 3 weeks. Thus, the leucopoietic response was not paralleled by an increase in antibody synthesis. It is of interest to note that the delay in antibody response observed in this experiment is quite similar to that following pantothenic acid or pyridoxine administration to previously immunized deficient animals(4).

The pronounced effect of a pteroylglutamic acid deficiency upon antibody response is comparable with that previously noted in a pantothenic acid deficiency state(1,3). The observation of Wright and Welch(10) that the utilization of pantothenic acid may be dependent upon the presence of pteroylglutamic acid suggests that the action of pteroylglutamic acid upon antibody synthesis may be mediated through its effect upon pantothenic acid metabolism. Further experimentation will be required to determine whether pteroylglutamic acid plays a direct or indirect role in the processes of antibody fabrication.

In agreement with previous observations (11-13), supplementation of the low niacin-tryptophan diet with either niacin or tryptophan produced a marked growth response. However, a normal antibody response was obtained only in those animals receiving added tryptophan. The efficacy of tryptophan in promoting both growth and antibody synthesis is explicable in terms of its ability to serve as a direct precursor of niacin(14) or niacin derivatives as well as its essential function in protein synthesis. On the other hand, the mechanism through which niacin affects growth under these conditions is not completely understood. In this connection, Krehl *et al.*(12) have observed that niacin improves the utilization of tryptophan. Niacin may also exert a "sparing" effect upon the requirement for tryptophan. Whatever the mechanism, it seems clear that niacin alone cannot satisfy the requirement for antibody synthesis on this basal diet. With the administration of niacin, it would appear that tryptophan is the limiting factor for antibody formation although apparently sufficient amounts of this amino acid are made available under these circumstances for growth purposes. The production of a more pronounced tryptophan deficiency state by employing a diet completely devoid of tryptophan would aid in clarifying the role of this compound in antibody synthesis. In the course of our studies on the relationship of nutritional state to antibody formation, we have occasionally encountered similar instances where a diet may be adequate for growth purposes and yet not for antibody synthesis. Thus, it would seem that the process of antibody synthesis is a more sensitive criterion of dietary adequacy than that of growth and, as such, may be used to advantage in the evaluation of nutritional state.

The role of vit. A in influencing susceptibility to infection has been the subject of numerous investigations(15-18). Studies (19-21) designed to determine the ability of vit. A-deficient animals to form antibodies following antigen administration have shown no impairment of this function. In the present experiments, however, the antibody titers of vit. A-deficient rats were definitely lower than those of control animals. The decrease, however,

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was of a lower order of magnitude than that encountered in pantothenic acid, pyridoxine (1), and pteroylglutamic acid deficiencies. It seems possible that the increased susceptibility of vit. A-deficient animals to disease processes may be attributed in part to their decreased antibody-synthesizing ability.

With respect to vit. D-deficiency, it is generally agreed that rachitic animals are no more susceptible to infection than controls receiving adequate doses of this vitamin(15). In addition, studies(20,21) of the ability of rachitic animals to produce antibodies following antigen injection have indicated that there is no impairment of this function. In agreement with these observations we found no significant difference between antibody levels of vit. D-deficient rats and those of control animals.

The experiments reported in this paper complete an extensive study of the effect of the following deficiency states upon the antibody response of the albino rats (Sprague-Dawley strain) to the antigenic stimulus of Group O, Rh positive human erythrocytes: pantothenic acid, pyridoxine, riboflavin(1), biotin, thiamine(2), pteroylglutamic acid, niacin-tryptophan, vit. B_{12}, vit. A, and vit. D. Identical immunological procedures were employed throughout these investigations. From our results it is possible to classify roughly the effects of these deficiencies upon circulating antibodies into 3 groups as follows: Group I. Severe impairment of antibody response—pantothenic acid, pyridoxine, and pteroylglutamic acid deficiencies. Group II. Moderate impairment of antibody response—riboflavin, thiamine, biotin, vit. A, and niacin-tryptophan deficiencies. Group III. No impairment of antibody response—vit. B_{12}, and vit. D deficiencies.

Summary. 1. Hemagglutinin production in response to inoculation with human erythrocytes has been investigated in pteroylglutamic acid, niacin-tryptophan, vit. B_{12}, vit. A, vit. D-deficient rats and their respective controls. 2. Severe impairment of antibody response was observed in the pteroylglutamic acid-deficient rats. Moderate impairment was demonstrated in vit. A and niacin-tryptophan deficiencies. Vit. B_{12} and vit. D deficiencies had no effect on antibody formation. 3. A rough classification of the effect of 10 deficiency states upon antibody production is presented.

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Relative Effectiveness of Pantothenic Acid and Panthenol in Stimulating Antibody Response of Pantothenic Acid-Deficient Rats.* (18837)

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Panthenol, (α,γ-dihydroxy-N-(-3-hydroxypropyl)-β,β-dimethyl butyramide) the alcohol analogue of pantothenic acid, was demonstrated by Pfaltz(1) to be approximately as effective as pantothenic acid in enhancing growth and curing pantothenic acid deficiency symptoms of the rat. Later Burlet(2) showed that panthenol was oxidatively converted to

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